Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

THE GASTRULATION OF THE EGG OF BUFO LENTIGINOSUS.

HELEN DEAN KING.

An examination of the literature dealing with the early development of the amphibian egg shows many conflicting observations and theories regarding the origin of the blastopore and the manner of formation of the mesoderm and notochord. It is evident, therefore, that more forms must be studied and the observations in some cases carefully repeated before any general conclusions regarding the origin of these structures can be drawn for the entire group.

The amphibian eggs that have been most studied are those of Rana, Triton, Axolotl, and Bombinator. The present paper deals with the early development of the egg of *Bufo lentiginosus* from the end of cleavage until the closure of the blastopore. The eggs of this amphibian, although very abundant and easily obtained, have been but little used either for observation or for experiment, owing, doubtless, to their small size and to their relatively deep pigmentation, which makes it very difficult to follow the fate of living cells.

METHOD.

In preparation for sectioning, eggs were killed in various fluids: picro-acetic, picro-sulphuric, formalin, and corrosive-acetic. By far the best results were obtained with corrosive-acetic (5 per cent acetic). Formalin (3–10 per cent) gives exceedingly good preparations for a study of surface structures, but it cannot be relied on to give histological details, as it usually produces numerous cracks in various parts of the egg. The sections were stained on the slide with the mixture of borax carmine and Lyon's blue, recommended in a previous paper (King, 10). With the use of this stain the nuclei appear red, the yolk blue, and the cell outlines are brought out with great clearness.

THE FORMATION OF THE BLASTOPORE.

The egg of *Bufo lentiginosus* probably contains a greater amount of pigment in proportion to its size than that of any other common amphibian. It is, therefore, very difficult to study the movements of individual cells before and during the formation of the blastopore, as has been done to some extent in the eggs of several other species of amphibians, where the pigmentation is less extensive and cell outlines can be readily determined.

In the egg of Bufo the pigmentation extends in all cases some distance below the equator, and I have frequently found eggs in which fully three-fourths of the surface was deeply pigmented before the appearance of the blastopore. Individual eggs, even from the same female, differ greatly in the amount of pigment they contain. As a rule, the pigment line extends farther down on one side of the egg than on the other, as seen in Fig. 1, agreeing in this respect with the frog's egg according to Schultze (24), Morgan and Tsuda (17), and Wilson (27).

Sections through an egg at the close of the blastula stage show a large segmentation cavity in the upper hemisphere. Its dorsal wall is formed, as in the frog, of three or four layers of small angular cells of uniform size. The cells forming the outer surface of the egg are almost completely filled with pigment granules, and a considerable amount of pigment is scattered throughout all the cells of the upper part of the egg. The yolk cells below the segmentation cavity are much larger, more rounded, and stain less intensely than the cells in the upper hemisphere. There is the same gradual increase in the size of the cells from the upper to the lower pole that other investigators have noted in the frog's egg.

The dorsal lip of the blastopore invariably appears some distance below the equator of the egg, but never in the middle of the lower hemisphere, as maintained by Houssay (8) for the axolotl and Jordan (9) for the newt.

If the egg has but the usual amount of pigment, the first evidence of the blastopore, in surface view, is a short, dark, almost straight line at the extreme edge of the black cells (Fig. 1). If the pigmentation is unusually extensive, the blastopore appears in its same relative position with respect to the lower pole of the egg but distinctly within the black cells. Whether there is, as the first step in the formation of the blastopore, a "lining up" of the ectoderm cells, as described by Wilson for the frog, I have not been able to determine.

Sections of an egg at the beginning of gastrulation show that the dorsal lip of the blastopore is formed primarily by a sinking in of several of the surface cells (Fig. 9). The shallow depression thus formed is rapidly extended and soon becomes a pronounced furrow (Fig. 10). The cells involved in this sinking in are all, without question, large yolk cells which are decidedly wedge-shaped and contain a considerable amount of pigment in their smaller ends turned towards the exterior (Figs. 9, 10, 11).

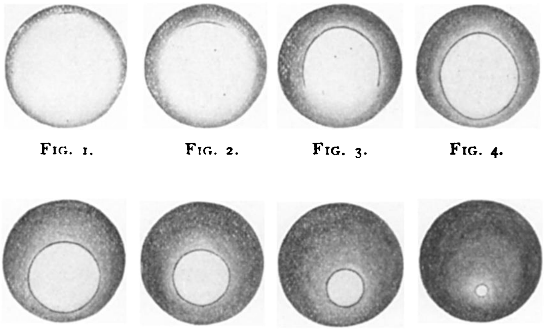
FIG. 1. FIG. 2. FIG. 3. FIG. 4.

FIG. 5. FIG. 6. FIG. 7. FIG. 8.

FIGS. 1-8. — Surface views of the lower hemisphere of an egg during the formation and closure of the blastopore.

After the lateral extension of the dorsal lip to form a crescent, a sagittal section through the blastopore shows that the furrow has deepened considerably and that its inner end is turned up towards the dark pole (Fig. 11). From this time on there is a marked difference in appearance between the cells forming the dorsal wall of the archenteron and those forming the ventral wall. The cells of the dorsal wall are small, angular, deeply pigmented cells, and they are apparently exactly like the small cells forming the upper surface of the egg, except that they do not contain quite as much pigment. The yolk cells which form the floor of the archenteron are three or four times larger than the cells of the dorsal wall, and they are more rounded and contain little if any pigment. The distinction between these cells is made more evident by the fact that with the combination stain used all the yolk cells appear decidedly blue, while the cells of the dorsal wall of the archenteron and, in fact, all the cells of the upper hemisphere, take a distinctly reddish tint. The cells at the anterior end of the archenteron are still wedge-shaped at this stage. They are intermediate in size between the cells forming the dorsal wall and those forming the ventral wall of the archenteron, and they stain like the yolk cells.

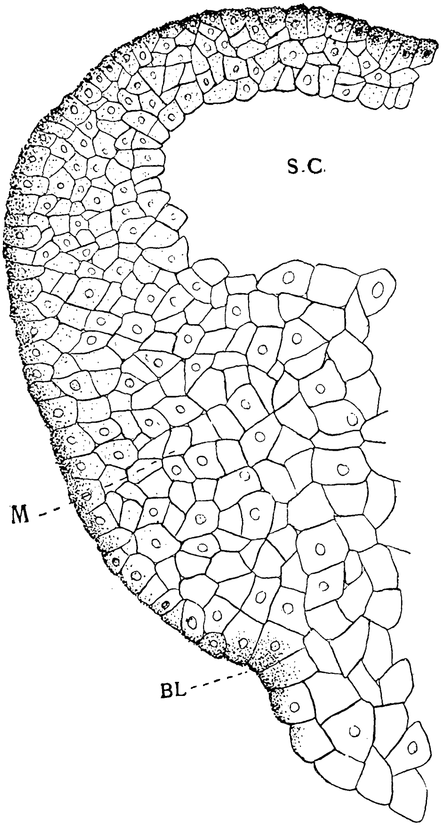

Fig. 9.— Part of a section through the median plane of the blastopore at the stage of Fig. 1, showing the depression of the yolk cells to form the dorsal lip. *S.C.*, segmentation cavity; *BL.*, blastopore; *M.*, cells which are to develop into mesoderm.

In opposition to most investigators, Moquin-Tandon (15), Houssay, Robinson and Assheton (20), and Marshall (14) maintain that the archenteron of the amphibian embryo is not formed by a process of invagination, but by a splitting between yolk cells which thus form the dorsal as well as the ventral wall of the archenteron. In a later paper, Assheton (1) states that "the formation of the primitive archenteron is by a process of splitting, and is the direct effect of the primary center of growth; whilst the continuation of the cavity produced by an overgrowth is the direct effect of the secondary center of growth."

That the archenteron in the egg of *Bufo lentiginosus* is not formed by a splitting between yolk cells is shown, I think, by a study of a series of well-preserved eggs during the early stages of gastrulation. The archenteron never appears "slit-like" at this time. On the contrary, its walls are usually some distance apart and its inner end is invariably rounded (Figs. 10, 11). Occasionally at the stage of Fig. 12, never earlier, I have seen an irregular cleft between the yolk cells at the anterior end of the archenteron which might, perhaps, be considered a forward extension of the archenteron, but such a cleft is usually seen only in badly preserved eggs where the cells are all more or less separated, and therefore I have always considered that it was artificially produced by the method used in killing and hardening the egg.

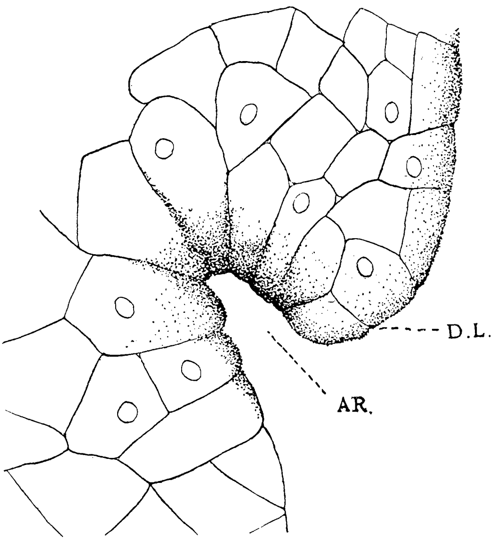

FIG. 10. — Part of a median section through the blastopore at a slightly later stage than Fig. 9, showing the character of the cells at the base of the blastopore furrow. *D.L.*, dorsal lip of the blastopore; *AR.*, beginning of the archenteron.

Jordan, Wilson, and Eycleshymer (4), among others, have watched the disappearance of individual surface cells under the dorsal lip of the blastopore in the early gastrulation stages of the living egg. These observations seem to me to afford conclusive evidence that invagination of cells plays an important rôle in the formation of the archenteron.

The presence of pigment in the inner ends of the cells forming the dorsal wall of the archenteron has been explained by Robinson and Assheton as follows: "The pigmented area is produced and extended by the deposit of pigment in the adjacent margins of a double row of yolk cells which eventually will form the boundary wall of the archenteron, and it also radiates from this area along the adjacent margins of the cells of each row. A slit-like space appears in the middle of the posterior portion of the pigmented area. This space first limits the dorsal lip of the blastopore, and then extends forward and ventrally, following the deposit of pigment, and separating the two rows of marginally pigmented cells from each other." During the early stages of gastrulation there is always found at the anterior end of the archenteron a marked accumulation of pigment that is extended around to include a few of the yolk cells in this region (Figs. 10–12). I have never found more than a few scattered pigment granules in the lower yolk cells except in this particular part of the egg, and, if the archenteron is formed by a splitting

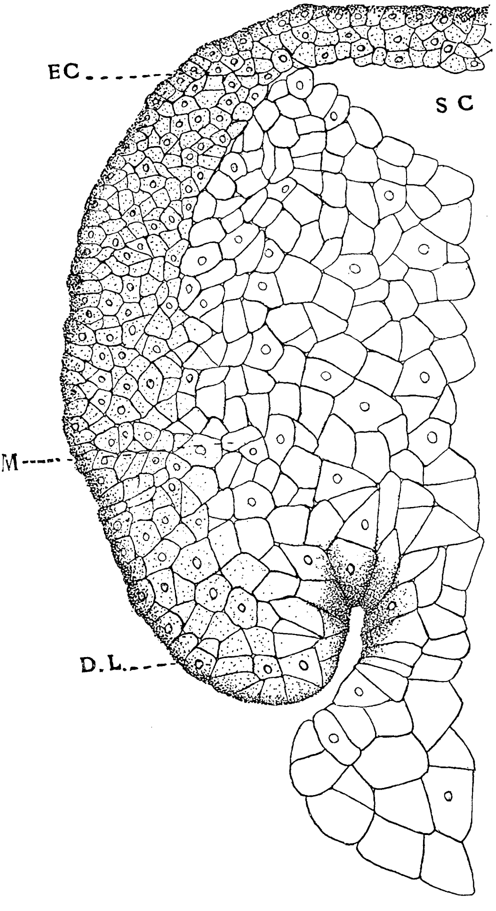

FIG. 11. — Part of a median sagittal section through the blastopore at the stage of Fig. 2. *EC.*, ectoderm. Other lettering as in previous figures.

between marginally pigmented yolk cells, then the pigment must at once almost entirely disappear on one side of the split and not on the other.

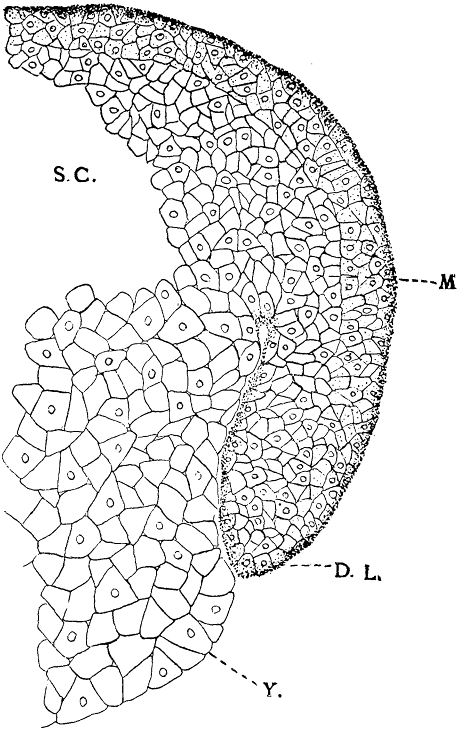

Fig. 12. — Part of a median sagittal section through the blastopore at the stage of Fig. 3. Y., yolk plug. Other lettering as in previous figures.

The pigmentation at the anterior end of the archenteron in the egg of Bufo is not confined to "a double row of yolk cells" anterior to the actual cleft. Well-marked lines of pigment may extend out in any direction, even at right angles to the line of advance of the archenteron, as seen in Fig. 13. From the time that the blastopore is first formed, the pigment in the cells of the dorsal wall of the archenteron is always collected around the cell walls, never around the nucleus. Rhumbler (22) considers this phenomenon to be due to the mechanical effect of pressure. The suggestion has been offered by Jordan "that the pigment marks physiological activity, and that the less heavily pigmented cells of the ventral wall of the archenteron owe their relative lack of pigment to more sluggish metabolism attendant upon less rapid cell division." It is certainly true that the large yolk cells in the egg of Bufo divide less frequently than the cells in the upper hemisphere, but there is no evidence that the deeply pigmented cells of the outer surface of the upper hemisphere or of the dorsal wall of the archenteron divide more rapidly than the cells that are found between them. If, therefore, Jordan's suggestion is correct, some kind of "physiological activity" other than that attendant upon rapid cell division must produce the pigmentation in these cells.

The extension of the lateral lips of the blastopore and the formation of the yolk plug have been so frequently described for other forms that a description of these processes is not necessary here, as they are apparently similar in all respects to those which take place in the frog. Following the method used by Pflüger (20), a number of eggs in the blastula stage were placed on a mirror in a shallow dish of water and the formation of the blastopore watched in mirror image. Figs. 1–8 show the appearance and the location of the blastopore at different times during the day, from 8 A.M., when the blastopore lip first appeared, to 6 P.M., when the egg began a rotation around its horizontal axis. There was no evidence to show that the position of the eggs was changed during the time they were kept under observation; on the contrary, one egg, in which a peculiar light spot was seen below the equatorial region, was watched particularly, and the spot was found to keep its same relative position until the rotation of the egg took place. These mirror images show that the dorsal lip of

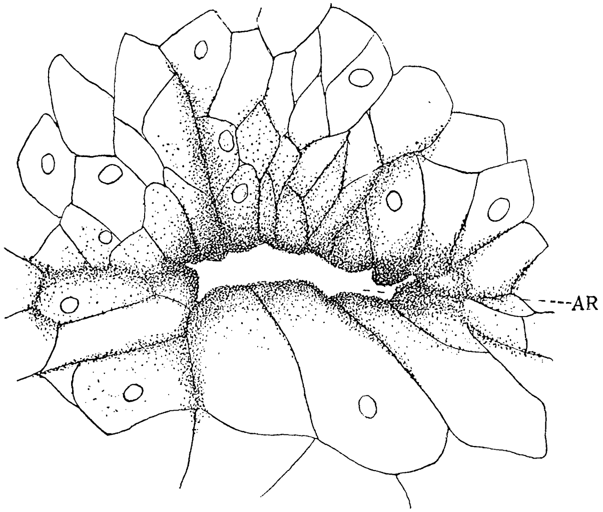

FIG. 13. — Part of a frontal section through the anterior end of the archenteron at the stage of Fig. 3, showing lines of pigment radiating out in all directions. *AR.*, archenteron.

the blastopore appears below the equator of the egg at the edge of the black cells (Fig. 1), and that the blastopore rim extends at a uniform rate in the form of a crescent around the lower hemisphere, until finally the yolk plug is formed (Figs. 2–4). Meanwhile, the dorsal lip of the blastopore has moved over the yolk, and seven hours after its first appearance has reached the center of the white hemisphere (Fig. 6). The closure of the blastopore lips takes place almost uniformly from all sides as a rule (Fig. 7), although occasionally the blastopore is somewhat oval when it is in the stage of Fig. 8. At or just before the stage of Fig. 8 a rotation of the egg around its horizontal axis takes place, thus bringing the dorsal lip of the blastopore back to its original position.

In its movement over the lower hemisphere the dorsal lip of the blastopore seems to pass through about 140° of the surface of the egg. There is apparently a slight variation in this respect in different eggs. This measurement is somewhat greater than that given by Morgan (16) and by Wilson (27) for the movement of the dorsal lip of the blastopore in the frog egg, and differs considerably from the measurements which have been given by other investigators. Roux (21) and Pflüger (19) estimate that the dorsal lip moves about 170°, while Kopsch (11) considers the movement to be but 75°; and this figure is further reduced by Assheton, who states that much of the so-called movement of the dorsal lip is only apparent and that the real movement is only from 60° to 70°.

THE FORMATION OF THE "GERM LAYERS" AND THE NOTOCHORD.

Observations regarding the origin of the endoderm, mesoderm, and notochord in different amphibian eggs are so conflicting that apparently the most plausible interpretation of them is to assume that there is absolutely no uniformity in the manner of formation of these structures even in species that are considered to be very nearly related to each other. Such an assumption, however, can scarcely be the correct one.

Goette (6), one of the early workers on the development of the amphibian egg, considered the mesoderm in the egg of Bombinator to arise from the splitting off of a layer of cells from the primitive entoblast, the layer thus formed extending as an unbroken sheet across the dorsal wall of the archenteron. The rest of the primitive entoblast was said to form the endoderm. According to Goette, a central chord of mesoderm in the mid-dorsal region of the embryo separates from the two lateral sheets to form the notochord.

A few years later Hertwig (7), after studying the development of the eggs of Triton and of *Rana temporaria*, came to the conclusion that the mesoderm in the amphibian egg arises in the vicinity of the blastopore "durch eine paarige Einfaltung des Entoblast schon zu einer Zeit wo die Gastrulaeinstülpung noch nicht ganz vollendet ist." The amphibian egg was thus brought into agreement with Hertwig's observations on the development of other vertebrates and gave additional support to his well-known cœlom theory. A few other writers — Balfour (2), Marshall (14), and Schwink (26) — agree with the results obtained by Hertwig.

In 1888, Schultze (25), from observations on *Rana fusca*, decided that "das mittlere Blatt, sowie die dorsale Urdarmwand entstehen aus dem Ektoblast und gehen an der dorsalen Urmundlippe alle drei Blätter in einander über; in den seitlichen und ventralen Theilen des Blastoporus setzt sich die Deckschicht des äusseren Keimblattes mit besonderer Klarheit in den Entoblast, die Grundschicht des Ektoblast ohne Unterbrechung in den Mesoblast fort." Perenyi (18), from observations on the egg of *Bombinator igneus*, Lwoff (13), from a study of Axolotl and various Anura, and Brauer (3), from investigations on two species of Gymnophiona, also came to the conclusion that the mesoderm is ectodermal in origin. These investigators, however, differ somewhat regarding the manner in which this process is supposed to take place.

Perenyi states that, as a result of a turning under, or "Duplikation," of the three-layered outer wall of the egg at the lips of the blastopore, "die äussersten Deckzellen der Blastula in ihrer ununterbrochenen Fortsetzung nach innen endlich die untersten

Zellenreihen bilden werden d. h. sie verwandeln sich in Entoderm, während die anderen zwei Zellenreihen der Blastula auch in ihrer Rückwindung beisammen bleiben und eine selbständige Schichte, das Mesoderm, bilden."

According to Lwoff, "die Einwanderung der Ektodermzellen und die Verschiebung der Entodermzellen zwei verschiedene und von einander unabhängige Vorgänge sind. Die Einwanderung von Ektodermzellen beginnt an der Stelle die das Hinterende des Embryo markirt; die Verschiebung der Entodermzellen beginnt im Gegentheil im vorderen Theile. Wie die weiteren Stadien lehren, bildet das Hineinwachsen der Ektodermzellen die zusammenhängende ektoblastogene Anlage der Chorda und des Mesoderms; die Verschiebung der Entodermzellen führt zur Bildung der Darmhöhle, die infolge des Auseinanderweichens der Entodermzellen entsteht."

Brauer's investigations show that there is a turning under of the outer surface cells, or "animal cells," at the posterior end of the germ disk and that by the forward growth of these cells under the upper animal cells a blind sac is formed. This blind sac becomes the posterior part of the archenteron, and at first its dorsal wall is composed of the animal cells invaginated from the surface. In subsequent development a layer of vegetative or yolk cells grows up from either side of the archenteron and forms a layer of endoderm beneath the invaginated animal cells which later become the mesoderm.

The origin of the mesoderm in the frog's egg has been described by Morgan (16) as follows: "The cells that are to form the mesodermal layer are present at the time when the dorsal lip of the blastopore has first appeared, and even just prior to that time." The innermost of the cells forming a ring around the equatorial region of the egg where the black and the white cells meet are the cells that later become the mesoderm. "These cells are carried up to the median dorsal line of the embryo by the closure of the blastopore. They will then be found forming a layer or sheet of cells that separates itself on the outer side from the thick layer of small ectodermal cells (that has been simultaneously lifted up) and that is separated on the inner surface, but not very sharply if at all, from the dorsal and dorsal-lateral walls of the archenteron."

In the egg of *Bufo lentiginosus*, when the blastopore first appears as a slight depression among the yolk cells just below the equator of the egg (Fig. 9), the cells which form the dorsal wall of the segmentation cavity are all small, somewhat angular cells which contain a considerable amount of pigment, particularly the cells which compose the outer surface of the egg. Near the equator the cells are found to be somewhat larger and to contain much less pigment, while in the lower hemisphere the cells are very large, and they have but a few scattered pigment granules. Up to this stage of development there is no visible separation of the cells into ectoderm, endoderm, and mesoderm. The smaller cells resulting from rapid cell division are found in the upper part of the egg and the larger cells, which divide less frequently, are grouped around the lower pole. The mass of cells marked *M* in Fig. 9 undoubtedly represent the region of the egg from which the mesoderm is formed. These cells are in no wise distinctive at this period, and their later development into mesoderm I consider to be due solely to their position in the egg during the formation and closure of the blastopore. The cells which are to become mesoderm form a layer around the egg at the equatorial region just inside the cells that are to become the ectoderm, as found to be the case in the frog's egg, according to Morgan. These mesoderm cells have many characteristics in common with the large yolk cells into which they grade, being larger, more rounded, and containing much less pigment than the small cells of the upper hemisphere. It seems probable, therefore, that they were originally produced by divisions of the yolk cells.

When the lips of the blastopore have extended so as to form a crescent in surface view (Fig. 3) a frontal section through an egg in the region of the blastopore (Fig. 14) shows that the dorsal wall of the archenteron is formed of several layers of small cells which have absolutely no distinction between them except that the cells of the outer layer, which are more regularly arranged, contain a much greater amount of pigment than the other cells. At the sides of the archenteron the innermost of these cells pass into the large yolk cells which form the floor of the archenteron. A median sagittal section through the blastopore at the same or a slightly earlier stage of development (Fig. 11) shows the beginning of the separation of the ectoderm from the inner cells of the egg. A tolerably regular cleft extends some distance around the sides of the egg, on a line usually with the lower edge of the dorsal wall of the segmentation cavity, sharply separating the layer of cells forming the outer wall of the egg from the cells within. The separation of the ectoderm does not extend as far down as the equator of the egg at this time, and it is some hours later before the ectoderm at the lips of the blastopore is distinct from the other cells. It is in the region of the blastopore that the union of the different layers persists longest, as other investigators have noted.

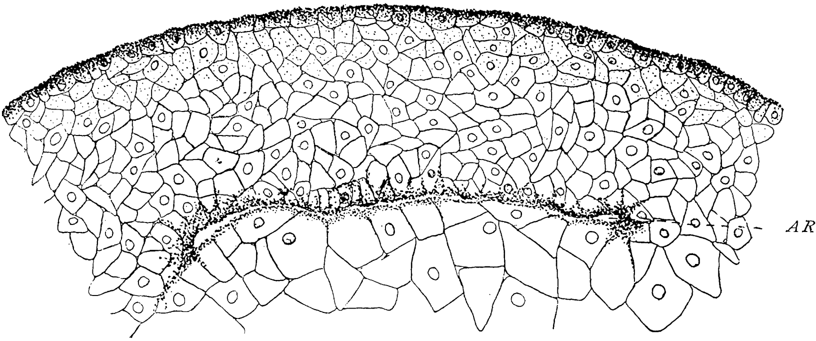

FIG. 14. — Part of a frontal section through the region just anterior to the blastopore at the stage of Fig. 3. *AR.*, archenteron.

At the stage of Fig. 3 the formation of the archenteron is well advanced, and a median sagittal section through the blastopore (Fig. 12) shows that the endoderm of the dorsal wall of the archenteron is formed of small, angular, deeply pigmented cells which, as far as I am able to determine, appear exactly like the small cells which form the outer surface of the egg. It seems probable that these cells once formed a part of the outer surface of the egg in the region just outside of the blastopore, and that they have been turned under the edge of the blastopore lip by a process of invagination, thus being changed into endoderm. Whether, in later stages of development, the endoderm of the dorsal wall of the archenteron receives additions from the yolk, as believed to be the case by Assheton and by Wilson, I have not been able to determine.

After the ventral lip of the blastopore has formed and the blastopore is beginning to close (Fig. 6), a division of the ectoderm into two layers is first noticed. A median sagittal section at this period (Fig. 15) shows an outer ectodermal wall composed of a single layer of small, deeply pigmented cells which are arranged very regularly. The inner ectodermal sheath is composed of two or three layers of much larger cells

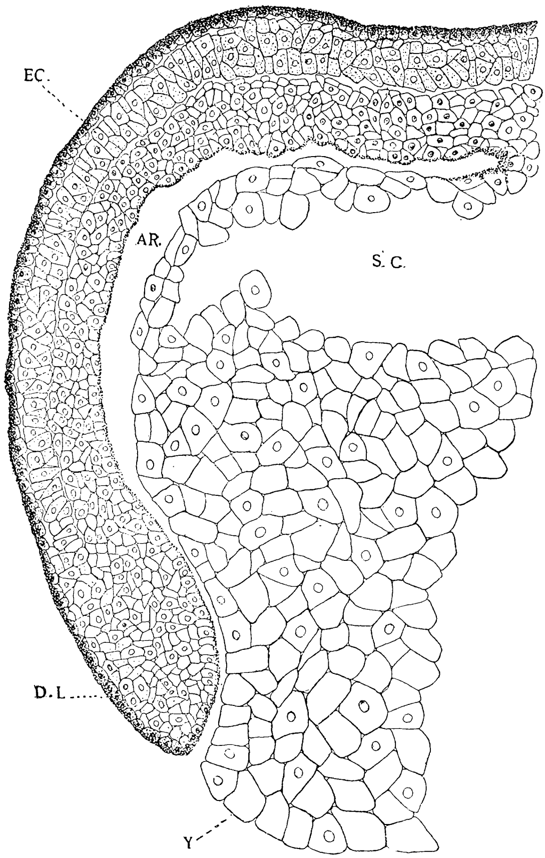

FIG. 15. — Part of a median sagittal section through the egg at the stage of Fig. 6. Lettering as in previous figures.

which are distinctly wedge-shaped and contain a comparatively small amount of pigment. The inner layer of ectoderm is sharply separated from the mass of small cells above the dorsal wall of the archenteron.

When, in surface view, the blastopore has reached the stage of Fig. 6, or in some cases even as early as Fig. 5, a splitting is seen in the mass of cells above the archenteron, and a single layer of endodermal cells is separated from the cells above.

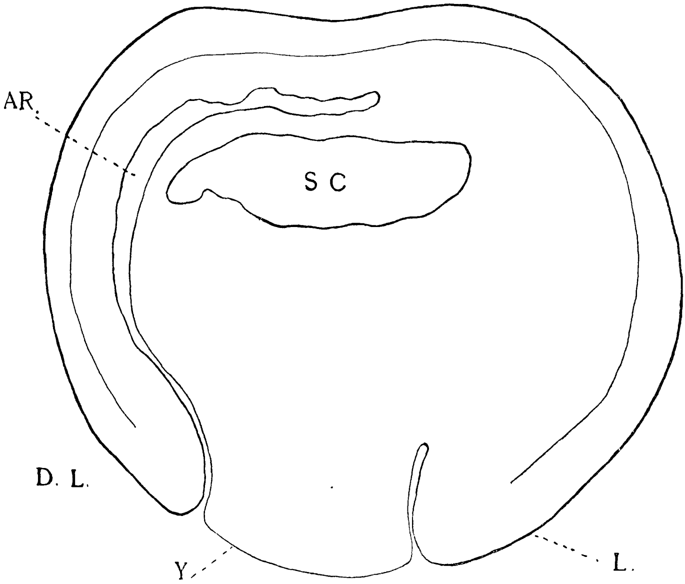

FIG. 16.—Outline of the entire section from which Fig. 15 was drawn. *L.*, ventral lip of the blastopore.

This process begins some distance in front of the region of the blastopore and gradually extends forward and backward from this point, thus completing the separation of the cells in the mid-dorsal region of the embryo into ectoderm, mesoderm, and endoderm.

When the blastopore has nearly closed (Fig. 8), a sagittal section through the middle of the dorsal lip (Fig. 17) shows that the endoderm cells of the dorsal wall of the archenteron (Fig. 17, *En.*) have flattened out considerably, and that they still contain much pigment, which is collected in the end of the cell bordering the cavity of the archenteron. Above the endoderm is the mesoderm layer (Fig. 17, *M.*), which varies in the number of its cells in different parts of the egg. Near the dorsal lip of the blastopore the mesoderm sheath consists of several layers of small cells which are not pigmented. Forward from this region the mesoderm gradually thins out until it is composed of only a single layer of rounded cells lying between the endoderm and ectoderm; then it gradually becomes thicker again, and near the ventral lip of the blastopore it appears very much as it does near the dorsal lip, being composed of several layers of cells.

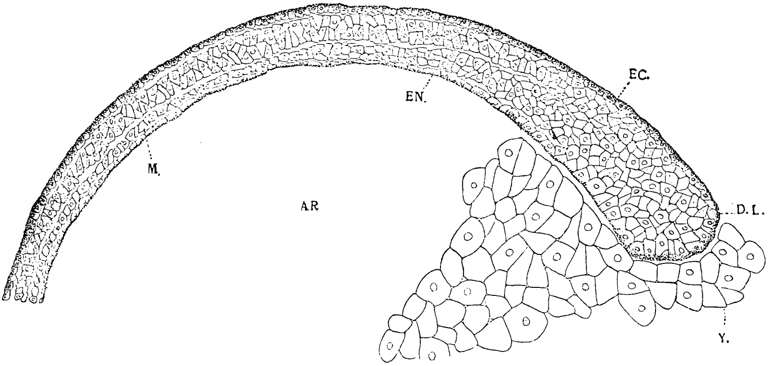

FIG. 17.—Part of a median sagittal section through the egg at the stage of Fig. 8. *EN.*, entoderm. Other lettering as in previous figures.

A frontal section through the middle of an egg at the stage of Fig. 7 shows a single layer of mesoderm over the dorsal region of the embryo, which gradually becomes several layers thick at the sides of the archenteron (Fig. 19), and below the archenteron passes directly into the yolk cells of the lower part of the egg. Still later stages during the closure of the blastopore show that the mesoderm is unquestionably extended at the expense of the yolk cells lying just beneath the ectoderm. Soon after the medullary folds have appeared the lateral sheets of mesoderm become fused on the ventral side of the embryo; thus a continuous sheet of mesoderm is formed around the embryo, except in the mid-dorsal region where the notochord has by this time been cut off from it. The extension of the lateral sheets of mesoderm by a process of splitting off from the outer yolk cells has been noted by Schwink (26) in *Bufo vulgaris*, by Scott and Osborn (23) and by Jordan in the newt, and by Morgan in the frog.

At the close of the blastula stage a very large segmentation cavity is found in the upper hemisphere of the egg directly under the black pole (Figs. 9, 11, 12). During the formation of the archenteron this segmentation cavity decreases considerably in size, and is pushed out of its position, coming to lie below the archenteron and being separated from it by only a thin layer of cells (Fig. 16, *S.C.*). During the closure of the blastopore the archenteron increases greatly in size, and there is then usually but a single layer of large yolk cells between it and the segmentation cavity (Fig. 19). When the blastopore is nearly closed, I have frequently found eggs in

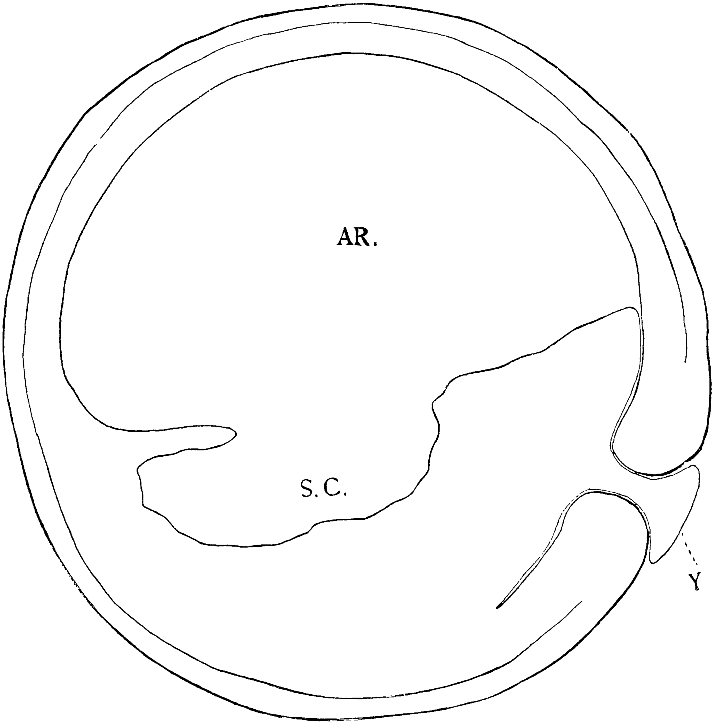

Fig. 18. — Outline of the entire section from which Fig. 17 was drawn.

which there appeared to be a direct connection between the two cavities which could not be due to poor preservation of the material (Fig. 18). It would seem, therefore, that in some cases the archenteron is extended at the expense of the segmentation cavity, as supposed to be the case by Kupffer (12) and by Marshall.

A series of frontal sections through the embryo at about the stage of Fig. 7 will show the various steps in the formation of the notochord. A section just in front of the dorsal lip of the blastopore (Fig. 20) shows that the mesoderm in this region forms a continuous sheath over the dorsal wall of the archenteron and that it is composed of three to four layers of small cells (Fig. 20, *M.*). About eight sections anterior to Fig. 20 there is a noticeable thickening of the mesoderm in the mid-dorsal region (Fig. 21), while on either side of this thickening the mesoderm usually consists of but two layers of cells. More anterior, the thickened part of the mesoderm layer is completely separated from the lateral sheets

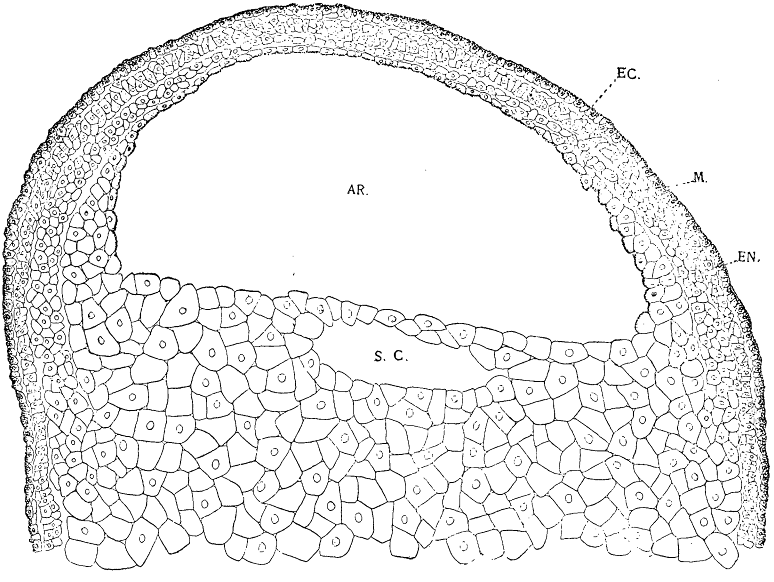

FIG. 19. — Part of a frontal section through the middle region of an egg at the stage of Fig. 7, showing the growth of the mesoderm at the expense of the yolk. Lettering as in previous figures.

as a distinct rounded structure, the notochord (Fig. 22, *N.*). For only a short distance at this stage of development is the notochord entirely cut off from the mesoderm; beyond there is the same thickening of the mid-dorsal portion of the mesoderm sheet as seen in Fig. 21. In the head region of the embryo the mesoderm is thinned out to a layer one or two cells deep which shows absolutely no thickening in the middle and appears very much as in Fig. 19. After the blastopore has closed, the notochord becomes extended forward in the head region and backward in the region of the tail.

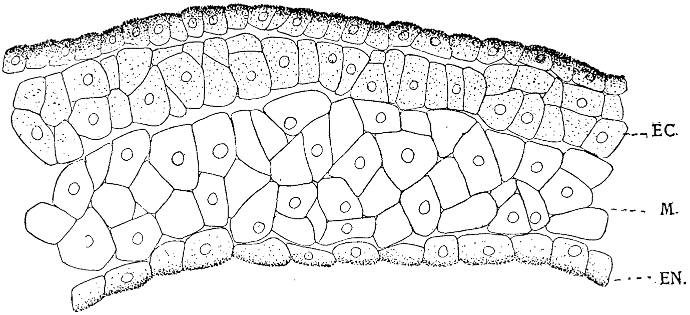

FIG. 20. — Part of a frontal section through an egg at the stage of Fig. 7, showing the mid-dorsal region of the embryo just in front of the blastopore. *EC.*, ectoderm; *EN.*, endoderm; *M.*, mesoderm.

Schultze, Goette, Lwoff, Brauer, Schwink, and Morgan also believe that the notochord is derived from the mesoderm; while other investigators consider that in the amphibians, as in other vertebrates, the notochord is endodermal in origin. Although Field (5) believes that in *Bufo vulgaris* the notochord arises

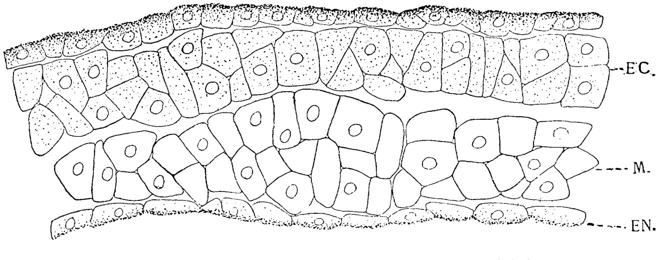

FIG. 21. — Part of a section from the same egg as Fig. 20 and slightly anterior to it. *EC.*, ectoderm; *EN.*, endoderm; *M.*, mesoderm.

from the endoderm, in the nearly related species, *Bufo lentiginosus*, the anterior part of the notochord is certainly mesodermal in origin. After the mesoderm layer has formed, the endoderm in the mid-dorsal region of the embryo is never more than a single layer of flattened cells. There is a closer connection between the mesoderm and the endoderm above the center of the archenteron than at the sides, but I have never found an egg in which these two layers could not readily be distinguished from each other. The endoderm cells are always much flattened at this period, and they invariably contain a considerable amount of pigment. The mesoderm cells and also the cells of the notochord are larger, more rounded, and contain but little pigment in comparison with the endoderm cells.

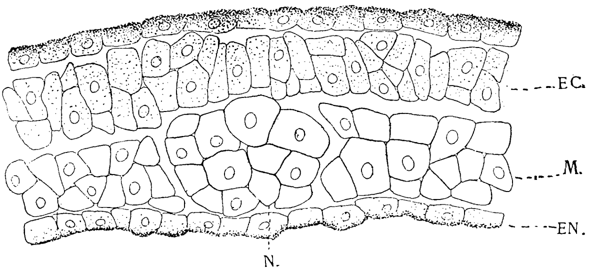

FIG. 22. — Part of a section from the middle region of the same egg. *EC.*, ectoderm; *EN.*, endoderm; *M.*, mesoderm; *N.*, notochord.

In conclusion, I wish to express my thanks to Prof. T. H. Morgan for advice and criticism during the progress of my work.

BRYN MAWR COLLEGE, BRYN MAWR, PA.,
December 16, 1901.

LITERATURE.

1. ASSHETON, R. On the Growth in Length of the Frog Embryo. *Quart. Journ. Micr. Sci.* Vol. xxxvii. 1894.
2. BALFOUR, F. M. Comparative Embryology. II. New York, The Macmillan Company, 1880.
3. BRAUER, A. Beiträge zur Kenntniss der Entwicklungsgeschichte und der Anatomie der Gymnophionen. *Zool. Jahrb.* Bd. x. 1897.
4. EYCLESHYMER, A. C. The Early Development of Amblystoma, with Observations on Some Other Vertebrates. *Journ. Morph.* Vol x. 1895.
5. FIELD, H. H. Bemerkungen über die Entwicklung der Wirbelsäule bei den Amphibien; nebst Schilderung eines abnormen Wirbelsegmentes. *Morph. Jahrb.* Bd. xxii. 1895.
6. GOETTE, A. Die Entwickelungsgeschichte der Unke. Leipzig, 1875.
7. HERTWIG, O. Die Entwicklung des mittleren Keimblattes der Wirbelthiere. I, II. Jena, 1881, 1883.
8. HOUSSAY, F. Études d'embryologie sur les vertébrés. *Arch. de Zool. Exp. et Génér.* Tome viii. 1890.
9. JORDAN, E. O. The Habits and Development of the Newt. *Journ. Morph.* Vol. viii. 1893.
10. KING, H. D. The Maturation and Fertilization of the Egg of Bufo lentiginosus. *Journ. Morph.* Vol. xvii. 1901.
11. KOPSCH, F. Beiträge zur Gastrulation beim Axolotl und Froschei. *Verh. Anat. Gesellschaft.* 1895.
12. KUPFFER, C. Die Entstehung der Allantois und die Gastrula der Wirbelthiere. *Zool. Anzeiger.* Bd. ii. 1879.
13. LWOFF, B. Die Bildung der primären Keimblätter und die Entstehung der Chorda und des Mesoderms bei den Wirbelthieren. *Bull. de la Soc. Imp. des Nat. de Moscou.* Bd. viii. 1894.
14. MARSHALL, A. M. Vertebrate Embryology. New York, Putnam, 1893.
15. MOQUIN-TANDON, G. Recherches sur les premières phases du développement des batraciens anoures. *Ann. des Soc. Nat.* Tome iii. 1876.
16. MORGAN, T. H. The Development of the Frog's Egg. New York, The Macmillan Company, 1897.
17. MORGAN, T. H., and TSUDE, UME. The Orientation of the Frog's Egg. *Quart. Journ. Micr. Sci.* Vol. xxxv. 1893.
18. PERENYI, J. Die Entwickelung der Keimblätter und der Chorda in neuer Beleuchtung. *Anat. Anzeiger.* Bd. iv. 1889.
19. PFLÜGER, E. Ueber den Einfluss der Schwerkraft auf die Theilung der Zellen. *Pflügers Archiv.* Bd. xxxii. 1883.

20. ROBINSON, A., and ASSHETON, R. The Formation and Fate of the Primitive Streak with Observations on the Archenteron and Germinal Layers of Rana temporaria. *Quart. Journ. Micr. Sci.* Vol. xxxii. 1891.
21. ROUX, W. Ueber die Lagerung des Materiales des Medullarrohres im gefurchten Froschei. *Anat. Anzeiger.* Bd. iii. 1888.
22. RHUMBLER, L. Physikalische Analyse von Lebenserscheinungen der Zelle. III. Mechanik der Pigmentzusammenhäufungen in den Embryonalzellen der Amphibieneier. *Arch. f. Entwickelungsmechanik.* Bd. ix. 1899.
23. SCOTT, W. B., and OSBORN, H. F. On Some Points in the Early Development of the Common Newt. *Quart. Journ. Micr. Sci.* Vol. xix. 1879.
24. SCHULTZE, O. Zur Entwickelung des braunen Grasfrosches. *Festschrift f. Kölliker.* 1887.
25. SCHULTZE, O. Die Entwicklung der Keimblätter und der Chorda dorsalis von Rana fusca. *Zeitschr. f. wiss. Zool.* Bd. xxvii. 1888.
26. SCHWINK, F. Ueber die Entwicklung des mittleren Keimblattes und der Chorda dorsalis der Amphibien. München, 1889.
27. WILSON, H. V. Formation of the Blastopore in the Frog Egg. *Anat. Anzeiger.* Bd. xviii. 1900.